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THE EVIDENCE OF PLAGUE INFECTION AMONG GROUND SQUIRRELS.

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The desirability of placing the diagnosis of plague infection among the ground squirrels of the Pacific coast upon as firm a foundation as possible is obvious. The presence among animals of a disease that may be transmitted to man, and especially when it has the epidemiological significance of the one we are discussing, is a matter of so much importance that every effort should be made to make the bacteriological work upon which the diagnosis is based so complete and careful that no flaw may be found. Executive authorities have a right to demand that prior to the inauguration of an extensive and costly campaign for the control or eradication of a disease all doubt as to its exact nature be removed.

After a considerable experience with both natural and experimental plague in ground squirrels, and having been called upon to make the clinical and bacteriological diagnosis in several cases of plague in persons who were probably infected from squirrels, it seemed that it might be of interest to show just what was the nature of the evidence upon which the diagnoses were based, omitting technical details.

The subject will be considered, first, in relation to ground squirrels, and, second, and somewhat less fully, in relation to human cases.

PLAGUE IN GROUND SQUIRRELS (CITELLUS BEECHEYI).

Gross lesions.—It is well known that in the majority of cases of plague in man and in laboratory animals there are certain well-defined and easily recognizable gross anatomical changes. These lesions are to a large extent specific, so much so that the experienced observer can usually make a safe and accurate diagnosis from the post-mortem findings alone in plague-infected human beings, rats, or guinea pigs. With these facts in mind, it will be readily understood that one would expect to find certain characteristic lesions in squirrels. At the federal laboratory our earliest experience in this line was with artificially inoculated squirrels. We found that plague in these animals was, as we expected it would be, uniformly manifested by certain well-marked gross lesions.

Not only did we inoculate ground squirrels with strains of the plague bacillus isolated in California from human beings, naturally infected rats, and naturally infected squirrels, but also with a culture that had been isolated originally in Asia. The object was to ascertain whether the reaction of the squirrels was the same to this foreign culture of the plague bacillus as it was to what might be spoken of as the domestic strains. The lesions were practically identical with those caused by the domestic strains.

This preliminary work with artificially infected animals was of the utmost value when we came to handle the large number of rodents that were sent in when the campaign was actively started against the squirrel during the summer of 1909. While the lesions in natural squirrel plague were by no means so uniform as they are in laboratory animals (rats and guinea pigs) or as they were in the artificially infected squirrels, still the disease is attended by definite lesions in every case. The possibility was always kept in mind that cases might occur in which no lesions were present and in which one would be compelled to rely upon the examination of stained smears to arouse suspicion. As a result of much work upon this point, I have come to the conclusion that the naked-eye examination alone will always afford one ample grounds upon which to base a probable diagnosis, and no case has come under observation in which infection was found in an animal that failed to present macroscopical evidence of the disease. In other words, we believe the disease in squirrels is practically always attended by certain marked gross anatomical changes, just as is true in the case of plague in other animals.

It is not my purpose to describe here in detail the lesions of squirrel plague, but it may be stated that they consist, in the majority of cases, of a bubo, often alone, but frequently associated with caseous or purulent lesions in the lungs, the liver, or the spleen, or occasionally in all of these organs. Our experience in the examination of squirrels has in some respects been most fortunate. About 2,000 squirrels were sent to the laboratory before the first naturally infected one was found. The examination of this large number of healthy animals gave the laboratory staff a thorough training in the appearance of the tissues and organs in the normal rodents and, having been trained in the appearance of induced plague in squirrels, prepared us to promptly recognize the gross lesions of the disease in naturally infected ones. I may state here, as a matter of general interest, that as compared with rats, squirrels present comparatively few pathological changes. Nephritis, tumors, abscesses, and certain other lesions that are fairly common in rats have been very rare in the squirrels we have examined. So much for the naked-eye appearances that aroused suspicion of a ground squirrel being plague infected. Let us now turn to evidence of a different and more convincing nature.

Bacteriological findings.—The first naturally infected squirrel found in America was sent to the Oakland laboratory of the service in the summer of 1908. It was examined there by Former Acting Asst. Surg. W. B. Wherry, who sent part of the tissue to the writer at the federal laboratory in San Francisco, the two laboratories conducting independent bacteriological examinations. This was in accordance with an order of Surg. Rupert Blue, commanding plague suppressive measures, who wished to have the diagnosis of every case of plague in man or in rodents confirmed by at least two bacteriologists working independently. This plan was followed in the case of all squirrels that were proven plague infected in 1908. Several of the cases, both human and rodent, were also verified by Dr. W. H. Kellogg, representing the California State Board of Health. During the current year (1909), as the number of infected animals was very large, this checking of results was dispensed with. Doctor Wherry[a] has published the results of the work in 1908, to which the reader is referred for a valuable discussion of squirrel plague. The report covers 4 infected squirrels from Contra Costa County and 1 from Los Angeles County.

When a squirrel is found that presents lesions which are regarded as suspicious of plague, stained smear preparations are made from the bubo or from other lesions and a search is made for organisms that correspond morphologically and tinctorially with the pest bacillus. In a considerable percentage of cases the examination of these smear preparations shows the presence of enormous numbers of organisms that are identical in appearance with *B. pestis* as we are acquainted with it from human and from rat cases of the disease. To the experienced observer the microscopical examination gives most valuable assistance and many times practically enables one to confirm a diagnosis. In other cases but few characteristic organisms are found, and in still others none at all are seen. In a large number of cases of natural squirrel plague the diagnosis has been made upon the gross lesions to which reference has been made in conjunction with the finding of large numbers of characteristic organisms in smears. However, it was made a rule, to which there were no exceptions, that no county was reported as furnishing plague squirrels until at least one squirrel from the county in question has been proven infected by animal inoculation. Indeed, with very few exceptions, at least one squirrel from each infected farm was thus verified. In other words, even the finding of what we regarded as characteristic lesions, and of typical bacilli in smears, was not regarded as sufficient to justify us in pronouncing the squirrels of a county infected until that evidence had been confirmed by the results of cultural investigations or animal inoculation. In this manner the diagnosis has been verified in about 150 squirrels, coming from 6 counties and covering an area of several thousand square miles.

The procedure in these cases was as follows: Tissue (usually bubo) from a squirrel that on account of gross pathological changes was regarded with suspicion, whether or not pest-like organisms were found in smears, was used for the purpose of inoculating one or more guinea pigs or white rats. In a few cases both guinea pigs and white rats were used. When the inoculated animals died, which was usually from about the fifth to the eighth day, a careful necropsy was made and a record showing exactly the lesions found was prepared and filed. As I have said before, the post-mortem appearances of plague in guinea pigs and in white rats are quite uniform and characteristic. When pathological changes were found that were regarded as those of plague in the inoculated guinea pigs or rats, the squirrel was reported as infected. In addition, cultures were made from the majority of the laboratory animals and the specific causative agent of the disease was isolated and studied upon various artificial media. In a few cases *B. pestis* was isolated in culture directly from the tissues of the naturally infected squirrel without the use of a laboratory animal. The culture derived directly from a squirrel or from a laboratory animal was grown first upon ordinary agar. Upon this medium the growth of *B. pestis* is in the form of small grayish translucent colonies, which when touched with an inoculating needle have the peculiar property of being sticky, so that a long filament may be drawn out when the needle is withdrawn. Unless the culture has these properties there is but little use to pursue the investigation further. Transplants were made; to broth in which flocculi are formed and certain other characteristic appearances noted; to 3 per cent salt agar, where the remarkable alterations in shape spoken of as involution forms are produced; and upon litmus milk, which medium is not affected, or at most is rendered slightly less blue than the control sample. The reactions of the organism upon other media need not be discussed here. The properties mentioned belong only to the pest bacillus. Indeed, in spite of the large number of organisms that have from time to time been designated as "pseudo-pest" bacilli, there are no organisms which in cultural and pathogenic properties are likely to be mistaken for *B. pestis.*

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[a] Journal of Infectious Diseases, vol. 5, No. 5, Dec. 18, 1908, pp. 485–533.

In a number of cases these pure cultures were used for the purpose of reproducing the disease in laboratory animals, and from these animals the infecting organism was again recovered in culture.

This then is the direct evidence relating to plague in the squirrel, with the exception of what I shall have to say in regard to the relation to antipest serum of the plague bacillus isolated from squirrels.

HUMAN PLAGUE OF SQUIRREL ORIGIN.

I have had under observation two cases of plague in hunters in which the history pointed indisputably to contact with squirrels as the source of the infection. In each instance an infected squirrel was found in the vicinity where the man had been hunting; in one the infected squirrel had been found several months before the human case developed, and in the other case infected squirrels were found as a result of a search made after the human case had occurred. In each of these cases the time between the handling of squirrels and the onset of symptoms was well within the ordinary period of incubation of plague. I have seen another case (the Los Angeles case) in which there was a clear history of the patient having been bitten by a *sick* squirrel that he had picked up a few days before he became ill. In a fourth case there was no history of association with squirrels, but an infected squirrel was found upon the same farm about the same time that the human case developed.

Two other cases were not seen clinically, but the diagnosis was made from the tissues submitted for bacteriological examination. In these two latter cases there was no history of contact with squirrels, but subsequently plague-infected squirrels were found in the vicinity of the place at which the patients had lived. I do not believe that there is any reasonable doubt that all of these patients contracted the disease from ground squirrels. There was no history of any association between the different cases; in fact, they all occurred in widely separated localities and at different times. At the time when these cases occurred there was no known plague in the country, excepting that among the ground squirrels. One of these cases almost fulfilled the conditions of an experiment. A young man (J. B.) who lived in Oakland, Cal., went squirrel shooting. He secured about a dozen of the rodents, which he brought home with him. Three days later he became ill, the illness following the usual course of plague, and the clinical diagnosis was confirmed by bacteriological methods. Several months prior to this we had had several plague-infected squirrels sent to the laboratory from the farm upon which the young man had shot his squirrels.

Each of the 4 cases that I saw personally presented the usual symptoms of plague, and the histories indicated that the other 2 cases followed the usual course of the disease. One of the cases[a] followed the ordinary course of plague during the early part of the attack, but became subacute, the patient dying on the sixteenth day. In each of the 6 cases the bacteriological examination was carried out in very much the same way as has been described under the discussion of the bacteriological findings in squirrels, and from each one the plague bacillus was isolated.

The question as to just how these persons were infected can not be answered at the present time. There was a history of a squirrel bite in one case; in another, a history of an abrasion on the hand while skinning a squirrel. Whether these 2 persons were actually infected in these ways can not be definitely asserted. There was no clue in the other cases as to the mode of infection. An interesting point is the fact that in each of these 6 cases the primary bubo was situated in the axillary region. Three of the 6 patients died and 3 recovered, making the mortality 50 per cent, which is just about the mortality for plague among Europeans in general and is approximately the mortality in the recent (1907–8) San Francisco epidemic, where the infection was presumably derived from rats.

The final criterion in the diagnosis of plague is the isolation of the specific microorganism that causes the disease, and, as I have said, this has been accomplished in all of the human cases and in a large number of the squirrel cases. There is no practicable application of the agglutination reaction to the diagnosis of plague. We have carried out some experiments upon animals with the object of determining the practicability of the use of an ophthalmic or a cutaneous reaction, but the results have not been encouraging. In conjunction with former Acting Asst. Surg. L. L. Schmitt we are at present working upon the application of the reaction of complement deviation, as applied to the diagnosis of plague, but we are not yet prepared to make any statement upon the subject.

RELATION TO ANTIPEST SERUM.

There remains one subject bearing upon the identification of a given organism as *B. pestis* that will be briefly treated. I refer to the protective power of antipest serum. Just how much weight should be given to evidence of this sort is too large a subject to be discussed here. It is evident that the results will depend in a measure upon the dose and the potency of the serum and the virulence of the organism under investigation. It may be assumed for our present purpose that if a serum protects against an organism, that organism is of the same species as the one used to provoke the production of specific antibodies in the serum. The results of all of the serum tests of the cultures of *B. pestis* isolated from squirrels and persons believed to have been infected from squirrels is set forth here.

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[a] Journal of Infectious Diseases, vol. 6, No. 5, Nov. 26, 1909, pp. 670–675.

Source of culture.	Test animal.	Dose of serum.	Day of death.	Lesions.	Day killed.	Lesions.
Squirrel 1	Guinea pig	5 c. c. antipest			(a)	
Do	do	5 c. c. diphtheria (control).	Fourth	Acute plague		
Do	do	None (control)	do	do		
Do	Wild rat (Mus norvegicus).	5 c. c. antipest			Eighth	None.
Do	do	do			do	Do.
Do	do	None (control)	Fourth	Acute plague		
Do	do	do	do	do		
Squirrel A	Guinea pig	3 c. c. antipest	Eleventh	Chronic plague.		
Do	do	None (control)	Fifth	Acute plague		
Los Angeles squirrel.	Wild rat (Mus norvegicus).	5 c. c. antipest			Fourth	Do.
Do	do	do			do	Do.
Do	do	None (control)	Third	Acute plague		
Do	do	do	Fourth	do		
Human (J. B.)	White rat	2 c. c. antipest			Fourteenth.	Do.
Do	do	None (control)	Third	Acute plague		
Squirrel 383	do	2 c. c. antipest			do	Abscess at site.
Do	do	None (control)	Fourth	Acute plague		
Squirrel 391	Guinea pig	2 c. c. antipest			Fifteenth	Purulent bubo.
Do	do	None (control)	Seventh	Subacute plague.		
Do	White rat	2 c. c. antipest			Fourteenth.	None.
Do	do	None (control)	Third	Acute plague		

[a] Alive and well tenth day.

In each instance the "protected" and the "control" animals were of approximately the same weight, and were inoculated with the same culture, in the same dose and in the same manner (cutaneous or subcutaneous).

As will be seen by a glance at the table, the antipest serum exerted a very high degree of protective power, usually saving the life of the "protected" animal.

General considerations.—As to the significance of plague among the squirrels of the Pacific coast, the subject is one about which only the most guarded opinion may be expressed, as our knowledge of the matter is very limited. It is my personal opinion that the number of human beings to be directly infected from squirrels will never constitute a large element in the mortality and the morbidity of the infected section. With the knowledge that the disease exists among the rodents, appropriate measures will reduce the risk of its spread to a minimum and enable municipalities to protect themselves against an infection of their rats from squirrels.

In conclusion I may say that the question has frequently been asked by honest and friendly critics as to whether the disease among squirrels was due to the same organism that caused plague among rats and among people. This question may safely be answered in the affirmative, and I would call especial attention to the following points:

1. The gross lesions in any case of squirrel plague are analogous to those seen in man and in rodents (rats and guinea pigs).
2. The lesions caused by a foreign (Asiatic) and a domestic (squirrel) strain of *B. pestis* are practically identical.
3. An organism has been isolated from the human and from the squirrel cases that is not to be distinguished by cultural or other methods from foreign strains of *B. pestis.*
4. Antipest serum (made in Paris) protects against the strains of *B. pestis* found in the human and squirrel cases discussed here.
5. Several cases of typical plague in human beings, the diagnosis of which have been verified by bacteriological methods, have been traced to squirrel infection as clearly as one can trace such things.